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Effects of the Basic Multicellular Unit and Lamellar Thickness on Osteonal Fatigue Life

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Abstract

A remodeling cycle sets the size of the osteon and associated lamellae in the basic multicellular unit. Treatments and aging affect these micro-structural features. We previously demonstrated decreased fatigue life with an unexplained mechanism and decreased osteon size in cortical bone treated with high-dose bisphosphonate. Here, three finite element models were examined: 1) a single osteon, as a homogeneous unit and with heterogeneous lamellae and interlamellae, 2) a control, interstitial-only tissue and 3) the osteon with cement line, set within the interstitial tissue. Models were loaded in simulated, sinusoidal bending fatigue. As osteon size was decreased, lamellar number and lamellar thickness were incrementally adjusted for each model. As hypothesized, lamellae within the larger type-1 models attained greater cycles to failure and the addition of an osteon to type-2 models (generating a type-3 model set) yielded increased fatigue life. However, as the osteon size was decreased, the potential for compressive damage nucleation was increased within the lamellae of the osteons versus the interstitium. Also, osteons with fewer, thicker lamellae displayed increased fatigue life. Osteonal microstructure plays a role in damage initiation location, especially when BMU size is smaller. Previous findings by us and others could partially be explained by this further understanding of increased probability for damage nucleation in smaller osteons.

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Conflict of Interest Statement

There are no known conflicts of interest, no significant financial support that could have influenced its outcome and no benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this work. Thus, the content is solely the responsibility of the authors and does not necessarily represent the official views of any agency or other group whom were not involved in study design, collection, analysis, interpretation of data, writing of the manuscript, or decision to submit the manuscript for publication. Further, the manuscript was approved by all authors and there are no other persons who satisfied the criteria for authorship but are not listed.

Keywords

Osteon; Lamella; Interstitium; Remodeling

Introduction

Bone remodeling effects tissue damage removal and mechanical property restoration via new tissue (Bajaj et al., 2014; Geissler et al., 2015; Lee et al., 2002; Nalla et al., 2003). Damage occurs primarily through fatigue from daily cyclic activities, presenting as microcracks within the bone microstructure (Diab and Vashishth, 2005; George and Vashishth, 2005; Nalla et al., 2003). The heterogeneous makeup of the bone's microstructure and lower-level hierarchy are generally attributed with the resistance of bone to fatigue and fracture. Remodeling restores heterogeneity, decreasing the local tissue age in the process (Abdel-Wahab et al., 2012; Ascenzi and Lomovtsev, 2006; Demirtas et al., 2016).

In a remodeling cycle the basic multicellular unit (BMU) first removes tissue. An osteoclast-filled cutting cone is followed by osteoblasts in a closing cone that generates a new osteon within the resorbed Howship's cavity. Within cortical bone the osteoblasts form full osteons by Type-1 collagen production in lamellar layers that produce a columnar structure. Each lamella is laid down during one cycle of growth with a size that can be affected by many factors (Bromage et al., 2009). Alterations in osteon structure and/or lamellae have been suggested to influence the load bearing and fatigue resistance of bone in large, long-lived animals, including humans (Ascenzi et al., 2016; Barth et al., 1992; Geissler et al., 2015; Pidaparti and Burr, 1992).

Our group found that osteon cross-sectional size, including wall-width (or diameter), and osteocyte density was smaller after 3-year, high-dose treatment with an anti-resorptive bisphosphonate, alendronate, in the rib of adult, female beagle dogs (Bajaj et al., 2014). Additionally, our results and that of another group indicate that in large animal models, long-term, anti-resorptive treatment with bisphosphonates decrease cortical bone tissue fatigue life measured *ex vivo* (Bajaj et al., 2014; Brock et al., 2015; Geissler et al., 2015). Bisphosphonates are designed to attenuate bone resorption. This aids in the maintenance of bone mineral density and reduces clinical fracture risk for many. Thus, the 14% smaller osteon area, corresponding to smaller osteon diameter, in our previous study is not surprising, given the restraint on the resorptive phase of the BMU expected with an anti-resorptive agent. An explanation for reduced osteocyte density is not so straightforward and may reflect a reduced proliferation rate of osteoblasts required to fill resorption spaces (Bromage et al., 2009). Osteocytes are believed to be the primary activators of resorption and recent *in vitro* data suggests that regulation by osteocytes may be a means by which bone formation and resorption are maintained in equilibrium (Bonewald, 2010; 2011). An alteration in osteoblast proliferation would be expected to also alter lamellar thickness. A recent study suggests that non-osteoporotic women with low-energy fracture exhibit significant reduction in lamellar width and average single osteon area (Ascenzi et al., 2016). The latter data support previous observations that secondary osteons formed at older ages are smaller than those formed at younger ages (Evans, 1976; Watanabe et al., 1998).

We hypothesized that smaller osteons may provide a partial mechanistic explanation for decreased tissue fatigue life. Specifically, a shift in damage nucleation sites with cyclic loading may result from an increase in relative interstitial volume and result in decreased tissue heterogeneity. Because the BMU's resorption cavity size determines osteon size and thus may alter lamellar thickness or number during refilling, we also investigated whether lamellar properties affect damage nucleation sites during cyclic loading. To explore these influences single osteon cortical units, based on the results of isolated, prepared osteon samples of Ascenzi et al. (1990), were modeled using finite element analysis (FEA), providing independent evaluation for the roles of osteon geometry and lamellar makeup toward damage nucleation.

Materials and Methods

Models were designed in ANSYS Parametric Design Language (APDL) (version 14.0, Academic Research) and run using an Intel Xeon CPU (E3-1240 V2 @ 3.40 GHz) with 16.0 GB RAM (Windows 7 Professional, 64-bit), with additional processing via a NVIDIA Quadro 2000 card (1 GB, 128 GDDR5). All models were meshed with 3-D, 20-node homogeneous (nonlayered) structural solid, tetrahedral elements. To test element size requirements, convergence testing was performed on the most complex of models, with cylindrical osteon and surrounding interstitial volume loaded in simulated 3-point bending. Element number ranged from 34,053 to 749,099 (Figure 1). The finest mesh (~750,000 elements) was chosen for subsequent models as maximum shear stress at the representative cement line node showed an asymptotic behavior.

For the osteons and complexes, the volumes representing individual lamellar and interstitial layers were fully attached at their boundaries to share regions and points that were used by ANSYS to guide the construction of geometry. Thus, the individual elements at the boundary of each layer (and the nodes that define the element geometry) interact as if layers are part of a continuous material, with bonded lamellar, cement line and interstitial regions and no contact-based analysis is performed. Elements within each layer were assigned separate, linear elastic isotropic material properties.

Three model types were designed: 1) a single osteon alone, as a homogeneous unit with a single elastic modulus and as a unit with each layered lamella containing a unique elastic property, 2) a homogeneous block of interstitium with a single elastic modulus, with and without an osteonal canal, and 3) a complex consisting of a layered osteon (type 1), with a cement line centered in a block of interstitium (Figure 2; Table 1). The single-osteon model was utilized for comparison with the results obtained in quasi-static, monotonic, 3-point bending studies conducted by Ascenzi et al. (1990). Comparison between models of single osteons with and without lamellar structure also provides an estimate for the degree to which lamellar structure influences behavior of the single osteon. Complexes (types 2 and 3) were utilized to explore the effects of osteon presence, wall thickness and lamellar makeup on potential for damage nucleation.

In previous studies, layers appearing dark by microscopy, due to plane-polarized light, are typically referred to as “dark lamellae” or by scanning electron microscopy (SEM) as “thick

lamellae.” We termed these “lamellae.” We termed “interlamellae” to refer to “bright lamellae” by polarized, light microscopy or “thin lamellae” by SEM (Ascenzi and Bonucci, 1976; Ascenzi et al., 2003; Ascenzi et al., 2016; Bhushan, 2004; Rho et al., 1999). Lamellar and interlamellar thicknesses ranged from 3–7 μm based on the average reported for osteons from a large range of mammals (Bromage et al., 2009). Unique, individual lamellar and interstitial properties were based on those reported by Hogan, 1992 and Rho et al., 1999 (Table 1). The Rho et al. (1999) data are based on single osteons with 8 “thick” lamellae with a gradation of highest to lowest moduli at innermost and outermost lamellae, respectively. These values agree with average lamellar and interstitial values reported by others (Ascenzi et al., 1990; Ascenzi et al., 2016; Barth et al., 1992; Bhushan, 2004; Bromage et al., 2009; Hengsberger et al., 2002; Hogan, 1992). The gradient is due to a degree of calcification that is higher near the osteon canal and lower near the cement line. “Thin” interlamellae have a considerably lower modulus and are within the range reported by Hengsberger et al. (2002).

The 14% decreased osteon area found in our previous study with bisphosphonate treatment was incorporated into the models by decreased osteon diameters and modifications of the lamellae (Bajaj et al., 2014). Thus, for all models, four osteon diameters were chosen: 100%, 96%, 93% and 90% (of 195 μm) to determine how osteon size affects damage nucleation sites (Table 2). Previous studies have demonstrated differences in osteon wall width (On.W.Wi does not include canal space) within these ranges (92% – 95%) for patients experiencing low-energy fractures (Ascenzi et al., 2016; Power et al., 2003). These osteons correspond to those in our study that are 4% – 7% smaller by diameter and thus, 8% – 14% smaller by cross-sectional area.

As the osteon size decreases two main changes may occur at the lamellar level, either lamellae become thinner or lamellar number decreases. Lamellar variations for each of the different osteon model diameters were designed to address the possible configurations based on the reported ranges for osteons as differences in lamellar thickness were suggested to be associated with increased fragility (low-energy) fracture risk (Ascenzi et al., 2016). Three basic variations were tested where there must be one fewer interlamellar compared to lamellar spaces: 1) 15 lamellae (8 lamellae, 7 interlamellae), 2) 13 lamellae (7 lamellae, 6 interlamellae), and 3) variable number of lamellae dependent on the osteon diameter (Table 2). For variations 1 and 2, the lamellar to interlamellar thickness ratio was 1:1. Thus, this thickness decreased proportionally with the decreased osteon diameter. For variation 3, the lamellar thickness was held as close to constant as possible, with removal of a lamellar/interlamellar pair as their thickness dropped below the minimum of 3 μm . In cases where small adjustments were required the lamellae were decreased in thickness. *In vivo*, lamellae form from the outermost to innermost, thus for 13-lamellar osteons, the modulus for the innermost lamella (lamella 1, Table 1) was not used, to represent the absence of this layer.

For the type-3 models the cement line was assigned a fixed thickness of 5 μm (Hogan, 1992). The interstitial dimensions for the largest osteon occupied approximately 50% of the square cross-section (approximately 237 $\mu\text{m} \times 237 \mu\text{m}$). The resulting osteon-to-interstitium ratios are within reported ranges (Ascenzi et al., 2016; Bajaj et al., 2014; Geissler et al., 2015). A 500 μm depth is the length of average, isolated, prepared single osteons observed

by Ascenzi et al. (1990) to avoid Volkmann's canals that would affect mechanical testing and add large discontinuities to models.

All loads and boundary conditions were also designed to replicate the three-point bending setup used by Ascenzi et al., 1990. Models were loaded in simulated three-point bending (Figure 3). Constrained and loaded patches (the area over which the load is applied in the model) were matched to Ascenzi et al. for single osteons ($50\text{ }\mu\text{m} \times 237\text{ }\mu\text{m}$ fixed patches at the bottom of each sample and a single centralized $80\text{ }\mu\text{m} \times 237\text{ }\mu\text{m}$ loading patch at the top). Loads were distributed over the nodes within the loading patch. Due to z-axis symmetry, computational time was reduced by use of models of half the total depth ($250\text{ }\mu\text{m}$ total depth).

Single-osteon load response was validated against the monotonic loading data of Ascenzi et al. (1990). Two, type-1 models were generated, one with a homogenized elastic modulus (reported for longitudinal osteons by Ascenzi et al., 1990) and one with the individual moduli reported by Rho et al., 1999 for a 15-lamellar osteon. Maximum displacement was recorded for a series of applied loads, and the resultant load verses displacement curves were compared with averages reported by Ascenzi et al. (1990).

Fatigue analysis was performed to obtain the probability of failure on each cycle at representative nodes throughout each mid-beam, cross-section (Figure 3g; Supplement 1). For the single osteons (type 1), six nodes were chosen corresponding to the outermost lamella, innermost lamellae and middle lamellar region. Within the beam cross section one node was selected in each of these regions, both above (top) and below (bottom) the Haversian canal. Four additional nodes were examined in the models that included interstitium (Types 2 and 3), corresponding to the cement line and interstitial tissue. The cyclic stress versus cycles-to-failure curve was input based on six stress amplitudes utilized in our previous study (Figure 4; Bajaj et al., 2014). The cycles-to-failure (S-N) curve provides the magnitude of a cyclic stress (S) against the cycles to failure (N). Stress amplitudes ranged from 45 – 85 MPa.

Results

Type-1 osteons were tested in a simulation of monotonic and fatigue loading. Maximum deformation data from the layered single-osteon model (100% On.Dm) agreed with the single, isolated, prepared osteon samples as tested previously by Ascenzi et al. (1990) in monotonic loading. The homogenized osteon model predicted greater deformation compared to all other models and the previously tested osteons (Figure 5). As expected, lamellae within the larger, layered, type-1 models exhibited greater fatigue life (number of cycles to failure) than in smaller osteons (Figure 6; Supplement 2; Ascenzi et al., 1990). In all cases damage nucleation occurred at the outermost lamella first, followed by the middle and then inner lamellae. Generally, osteon models with 13 thicker lamellae had less probability to nucleate damage than their 15 lamellae counterparts, regardless of osteon size.

The addition of the osteon components to a homogenized interstitial unit increased fatigue life for nearly all nodes of interest through the cross-section (Figure 7). The one exception

was at the top interstitial node for the 100% osteons, where the fatigue life for the osteon-containing complexes and homogeneous interstitium with osteonal canal nearly overlapped. The addition of the osteonal canal nearly doubled the fatigue life versus that of the solid interstitial beam. More markedly, fatigue life increased 10-fold compared to the solid interstitial beam (and in some cases, the interstitium with osteonal pore) with addition of the lamellar components and cement line. The increase was apparent regardless of the number or thickness of the lamellae, though certain combinations of osteon and lamellar size had greater effect.

For the complexes, as osteon size decreased, the relationship between lamellar number and fatigue life was generally similar to that observed with the single osteons alone. Damage nucleation initiated on the compressive side of the beam in all cases. Tensile damage was only observed for the bottom interstitial node of 100% osteon complexes at the highest stress amplitude (85 MPa). As the osteon size dropped below 96%, the osteons with fewer, thicker lamellae (13 lamellae at 90% and 93%) tended to have the least probability for damage nucleation (Table 2; Figure 7).

The complexes with the largest osteons tended to have overall greater probability for damage nucleation throughout their cross-sections compared to those with smaller osteons. However, as osteon size decreased to 93% diameter (14% decreased osteon area, Table 2), there was a shift in the relative number of fatigue cycles when comparing the interstitium and outermost lamella. For models containing smaller osteons, the fatigue life at the interstitial node increased, with relative decreases in the fatigue life of the outermost lamella. The net effect of this shift was a decreased probability of damage nucleation in the interstitial regions, with a concomitant increase in the probability of nucleation in the osteonal features as the osteon size decreased. This site-specific shift in potential for damage nucleation was more markedly apparent when fatigue behavior was plotted as normalized against that of the canal-containing interstitial beam (Figure 8).

Discussion

Certain alterations in osteon architecture associate with decreased bone quality and increased fracture risk (Ascenzi et al., 2016; Bajaj et al., 2014; Barth et al., 1992; Demirtas et al., 2016; Diab et al., 2005; Geissler et al., 2015; George and Vashishth, 2005). For example, canal size increases in osteoporosis leading to more porous tissue (Barth et al., 1992). While bisphosphonate treatment inhibits increased porosity, and slows the loss of bone mass, long-term exposure has demonstrated alterations in bone quality (Bajaj et al., 2014). We recently demonstrated that osteon cross-sectional size, including wall-width was smaller after 3-year, treatment with a high-dose of anti-resorptive bisphosphonate in adult, female, beagle dogs and was associated with reduced fatigue life of rib bone tissue beams (Bajaj et al., 2014). Previous studies, including recent examination of iliac crest biopsies, have suggested that non-osteoporotic women with low-energy fractures exhibit significant reduction in average single osteon area compared to age-matched controls, and in the absence of bone disease or porosity differences (Ascenzi et al., 2016; Power et al., 2003). This current FEA study aimed to determine the role of smaller osteon size in the ranges reported in these large animal and human studies, specifically diameters of 92 – 95%,

corresponding to smaller average osteon area by ~14% compared to controls (Table 2). We also explored the effects of associated decreased lamellar number and thickness on the site of potential damage nucleation during fatigue loading.

The observation of *decreased* probability for damage nucleation in osteons with fewer thicker lamellae, particularly as the osteon size *decreased*, may indicate another optimization of lamellar properties based on resorption pit diameter that determines osteon size (Figure 8). Damage nucleation site and type associate with patient (and potentially tissue) age (Frost, 1987a, b; Nobakhti et al., 2014; Skedros et al., 2005). Further, the width of lamellae in both the inferior region of the femoral neck and in iliac crest biopsies of non-osteoporotic, by DEXA BMD, women have been shown reduced in low-energy, fracturing individuals (Ascenzi et al., 2016; Power et al., 2003). The biological mechanism behind controlling new lamellae and osteocyte deposition could be hypothesized to include a surface area effect on the intensity of osteocyte signaling at the newly formed resorption canal when the cement line and first lamellae are being established to support pre-osteoblasts (Martin, 2000; Reznikov et al., 2014).

Our data indicate that even a small decrease in osteon diameter may move the probability for damage nucleation toward the components of the osteon, and away from interstitial areas, especially in the case of thinner lamellae. This influence would represent perpetuation of the formation of smaller osteons and potentially limit the amount of tissue that is removed over multiple cycles of remodeling activation. Upon activation, resorption and formation are maintained in equilibrium as part of the intricate cross-talk between the bone cells (Bonewald, 2010; 2011). In the case of an anti-resorptive drug, the initiation of smaller osteon size is conceivably due to the limits on the amount of bone tissue that is resorbed on each activation cycle. Ultimately, this repeated activation of remodeling near the cement line would increase the interstitial volume, potentially promoting decreased heterogeneity of damaged, older, interstitial bone volume and increasing fracture risk.

The assumptions made for any model limit applicability to “real world” situations and we attempted to make them based on previous data from our own and other work. All this previous work was on devitalized samples and thus results from our current work also cannot be expected to recapitulate what occurs in living bone. Our assumptions included assignment of linear elastic isotropic properties in an idealized geometry including boundary conditions, approximation of sinusoidal loading and use of a single canine S-N curve for all trials. Idealized geometry precludes the initiation of damage at microstructural features smaller than the thickness of a single lamella. This includes osteocyte lacunae that microdamage has been observed around, as microcracks may begin at the lacunar apices and be influenced by spatial orientation in combination with collagen and apatite organization (Ascenzi et al., 2008; 2012; Reilly, 2000). Damage nucleation at this level was not possible as lacunar structures were not included in the current model geometry. Additionally, at these lower levels of hierarchy cortical tissue is known to be highly anisotropic. Differences in the collagen orientation and the osteocyte lacunae-canalicular system (pores in the lamellar and interlamellae in which the osteocyte sensing network resides) contribute to anisotropy (Ascenzi and Bonucci, 1967; Prendergast and Huiskes, 1996). In our previous study a decreased osteocyte lacunar density was observed, and future models that consider lacunar

regions and Volkmann's canals may further improve upon our single-osteone model (Ascenzi et al., 2008; Bajaj et al., 2014; Vaughan et al., 2013). Further, non-idealized osteons, including unfinished ones with scalloped Haversian canals, that are sometimes observed on histology and thought to be due to an exhaustion of the osteoblast pool required to complete the work of refilling, should eventually be included. Finally, while our goal was to simulate fatigue loading, we have only monotonic data to validate our model against. Our fatigue results only suggest where nucleation of damage is likely to occur because fatigue to failure is a complex process that also involves propagation and coalescence of damage post nucleation. Thus, these results may not translate to a continuum where there are multiple osteons, fragments of osteons, osteons at various stages of the remodeling process, etc. We cannot conclude from the current simulation that fracture risk will be altered with different sized osteons. Lack of uniformity in higher-order models may improve fatigue resistance through increased interface heterogeneity, and this should be explored with multi-osteone models and additional validation experiments.

The results of this work permit us to hypothesize the existence of a mechanism for shifting sites of damage nucleation from the interstitial tissue to the osteonal features as the osteon size decreases. The trends indicate that the location of probable initial damage nucleation is less likely to occur in the interstitial tissue as the osteon size is decreased (Figure 8). This is most evident with the 15-lamellae model and least evident with the variable-lamellae model, although the trends are still apparent. The results also indicate that an initiation event, either decreased remodeling due to anti-resorptive treatment or the low turnover of aging to decrease osteon size, is first required. However, even a small size reduction may perpetuate decreased remodeling. The shift in nucleation site may present an additional means by which osteon size is limited, by concentrating damage within smaller osteons and thus focusing resorption primarily within these regions near the existing blood supply. Osteonal changes may be useful markers for monitoring patient bone quality and tailoring treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations (Dempster et al., 2013)

FEA	Finite Element Analysis
BMU	Basic Multicellular Unit
On.W.Wi	osteon wall width (µm)
On.Dm	osteon diameter (µm)
Lm.N	lamellar number
Ir.Lm.N	interlamellar number

Lm.Th lamellar thickness (μm)
Ir.Lm.Th interlamellar thickness (μm)

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Figure 1.

Convergence in maximum shear stress at the top cement line node (chosen as a representative location) as element number increased. An asymptote was approached as the number of elements utilized in the final models was approached (~ 750,000).

Figure 2.

Representative osteon model types of geometry: 1) Single osteons, both homogeneous and layered, 2) Homogeneous interstitium, without and with canal and 3) Layered osteon with cement line (outermost ring) and interstitium. Inset (reproduced with permission from Bajaj et al., 2014): Histology of an osteonal cross-section. The white and black arrows indicate the cement line and osteocytes residing between lamellar layers, respectively. Osteon and Haversian canal diameters were based on the average diameters found by Ascenzi et al., 1990 (approximately 195 and 46 μm , respectively). Increased canal diameter in the form of increased porosity has a known, large and negative influence on the mechanical properties of single osteons and cortical bone tissue (Barth et al., 1992). Thus, canal diameter was held constant for all trials to study the influence of osteon wall thickness and makeup of lamellae. Note that elements are hidden in model images. Thus, the lines indicate geometric features such as the lamellae, cement line and canal.

Figure 3.

Model loading (red) and boundary conditions (blue). a–c) The osteon, free of interstitium, was based on and validated with the three-point bending data of Ascenzi et al. (1990). d–f) Osteon-interstitial complex. c and f indicate regions for which fatigue life was measured. g) Complex cross-section with labeled nodes of interest. The Haversian canal is at the center. h) The sinusoidal loading was simulated as a ramped load at 2 Hz to approximate the sinusoidal loading employed previously (Bajaj et al., 2014).

Figure 4.

Stress amplitude (MPa) versus cycles to failure curve used as input for fatigue interpolation.

The maximum and minimum stress amplitudes were interpolated from previous S-N data (which spanned 45 – 85 MPa) to set absolute cutoffs for node survival (Bajaj et al., 2014).

Figure 5.

Comparison of the homogenized and layered, type-1 osteon models to the average response of the single machined osteons tested by Ascenzi et al. (1990).

Figure 6.

Fatigue life (x axis) at select nodes (middle and inner lamellae) for the type-1 models (without interstitial tissue) for each stress amplitude (y axis) and varying outer diameters. Outer lamellae failed at or before 100 load cycles and thus are not shown. Top) Fatigue life for the middle lamella. Bottom) Fatigue life for the inner most lamella.

Figure 7.

Fatigue life (x axis) at select nodes of interstitium (top), cement line (middle) and outer lamellae (bottom) for the type-2 models (homogeneous interstitium, both without and with canal) and type-3 models (interstitium plus osteon at each osteon size), at each stress amplitude (y axis). Nodes of interest within the osteon, except that of the top outermost lamellae (e.g. the middle and inner lamellae) tended toward fatigue lives greater than the 1,000,000-cycle maximum. Thus, they are not included. Note that the major effect on fatigue life at most nodes came from osteon size, except at the outer lamella where the number and thickness of lamellae influenced the probability of damage nucleation, especially as the osteon diameter became smaller.

Figure 8.

Relative fatigue life for each region and relative likelihood of damage nucleation within the interstitium as a function of osteon diameter. Note that the variable lamellar models that have 15 lamellae at 100% and 96% sizes and 13 lamellae at 93% and 90% sizes closely follow the model subtype that corresponds to their number of lamellae at each osteon size. Top) Average log difference in fatigue life (y-axis) between the type-2 model interstitium with Haversian canal and type-3 models diameter (x-axis). Bottom) Relative probability for initial damage nucleation in the interstitium versus the cement line (solid bars, red) and outer lamellar (outlined bars, green) regions. Differences in the graph (top) were normalized to the largest difference between cement line and interstitium (15 lamellae, 96%). Thus, greater positive values indicate a higher relative likelihood of initial damage nucleation in the interstitium and negative values indicate an even lower likelihood of damage nucleation in the interstitium with respect to the other regions (cement line or outer lamella). The relative likelihood of initial damage nucleation in the interstitium decreases as the osteon size decreases.

Table 1

Moduli for single osteon, complex and homogeneous interstitial models.

Structure	Elastic Moduli (GPa)	Study	Method	Poisson's Ratio
Lamella			Nanoindentation (Nano Indenter II, MTS Systems Co., Oak Ridge, TN)	
1 (inner)	22.5			
2	21.2			
3	21.0			
4	21.1	Rho et al., 1999		0.17
5	20.1			Budyn and
6	21.8			Hoc, 2007
7	20.4			
8 (outer)	19.4			
Interlamellae	6.85	Abdel-Wahab et al., 2012	Sabelman et al., 1997 determined the Poisson's ratio	0.49
Interstitialium	26.8	Rho et al., 1999	Nanoindentation (see above)	0.15
Cement Line	6.00	Hogan, 1992	Half the modulus of cortical bone	0.30
Av. Single Osteon	2.31	Ascenzi et al., 1990	Calculated from monotonic 3-point bending (Budyn and Hoc, 2007)	0.17
2X Av. Single Osteon	4.62			
3X Av. Single Osteon	6.93			

Table 2

Properties of the lamellar variants of the osteon models, including percent of **original (100%) diameter**, cross-sectional area, wall width, lamellar number and thickness.

<i>Model</i>				
<i>On.Dm (%)</i>	100	96	93	90
<i>On.Area (%)</i>	100	92	86	81
<i>On.W.Wi (%)</i>	100	95	91	87
15 lamellae				
<i>Lm.N</i>	8	8	8	8
<i>Ir.Lm.N</i>	7	7	7	7
<i>Lm.Th (μm)</i>	4.96	4.70	4.51	4.31
<i>Ir.Lm.Th (μm)</i>	4.96	4.70	4.51	4.31
13 lamellae				
<i>Lm.N</i>	7	7	7	7
<i>Ir.Lm.N</i>	6	6	6	6
<i>Lm.Th (μm)</i>	5.73	5.43	5.20	4.98
<i>Ir.Lm.Th (μm)</i>	5.73	5.43	5.20	4.98
variable # of lamellae				
<i>Lm.N</i>	8	8	7	7
<i>Ir.Lm.N</i>	7	7	6	6
<i>Lm.Th (μm)</i>	4.96	4.48	4.96	4.96
<i>Ir.Lm.Th (μm)</i>	4.96	4.96	5.48	4.99